REMARKS

Upon entry of the present amendments, 24-26, 28-30, and 32-40 are pending. The instant amendments are fully supported by the as-filed specification. As such, no new matter has been added.

Claim Rejections -- 35 U.S.C. § 103

Claims 24-40 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over United States Patent No. 5,968,546 ("Baur") in combination with Lenoir et al. ("Lenoir") and Lenoir-Viale et al. ("Lenoir-Viale") According to the Examiner, "Baur teaches all of the limitations of the cited claims with the exception that Baur teaches that the plucked hair is not cultured in toto but instead is subject to dissection and the hair bulb and infundibular parts are removed and the follicles are subsequently cultured." (Office Action at page 2). The Examiner also contends that Lenoir and Lenoir-Viale disclose that the further dissection of the plucked follicle by cutting off the soft end bulb and subsequently dissecting the follicle is done for matters of convenience rather than criticality to the production of epidermal or skin equivalents. (See Office Action at page 3). Applicants traverse.

Claims 27 and 31 have been cancelled, without prejudice or disclaimer. Thus, this rejection, as it applies to these claims, is moot and should be withdrawn.

In making an obviousness determination, the United States Patent and Trademark Office ("USPTO") is obligated to determine the scope and content of the prior art; to ascertain the differences between the prior art and the claims in issue; to resolve the level of ordinary skill in the art; and to evaluate evidence of secondary characteristics. See *Graham v. John Deere*, 383 U.S. 1 (1966). Moreover, objective evidence or secondary considerations are relevant to the issue of obviousness, and they **must** be considered by the USPTO, whenever they are present. See MPEP § 2141; *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530 (Fed. Cir. 1983); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367 (Fed. Cir. 1986); and *Rosemount, Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540 (Fed. Cir. 1984) (noting that objective evidence

and "real world" facts are entitled to great weight in a case). In addition, in order to establish a *prima facie* case of obviousness, three criteria must be met: 1) there must be some suggestion or motivation to combine the teachings of the cited references; 2) there must be a reasonable expectation of success; and 3) the prior art reference(s) must teach or suggest all of the limitations of the claimed invention. See MPEP § 2143; *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

Applicants have herewith amended independent claim 24 so that, in addition to requiring the use of an intact hair follicle, it now also specifies that the primary and organotypic culture media contain human serum in a concentration of less than 5% and that the culture density of the keratinocyte precursor cells are seeded at a density between 3×10^4 cells cm^2 and 1×10^5 cells/cm^2. The combination of these limitations in the claimed methods is neither taught nor suggested by the combination of Baur, Lenoir, and Lenoir-Viale and produces a different epidermal or skin equivalent.

Moreover, the claimed methods for the selection of keratinocyte precursor cells from the outer root sheath of hair for subsequent use in compositions for healing skin defects are nonobvious improvements of the methods disclosed by Baur. To this end, Applicants submit herewith the declaration of Dr. Alain Limat, one of the named inventors of the instant application, which describes "real world facts" that demonstrate the nonobviousness of the claimed invention. (Limat Decl. ¶ _).

First, Dr. Limat discusses the advantages of culturing intact hair follicles (as claimed herein) rather than dissected hair follicles (as disclosed in Baur). (See Limat Decl. ¶ 5-6). According to Dr. Limat, the culturing of intact hair follicles makes the production of the epidermal or skin equivalents simpler, less time consuming, less expensive, and less labor-intensive because the methods of the instant invention involve fewer preparation steps than the methods disclosed in Baur. (See Limat Decl. ¶ 6). Thus, contrary to the Examiner's contention, the removal of the hair bulb and infundibular parts in Baur is not merely a matter of convenience. Prior the Applicants' invention, it was the standard methodology was to remove these parts of the hair follicle prior to culturing. (See specification, page 3, lines 18-21; Limat Decl. ¶ 5). As noted by Dr. Limat, Applicants were the first to realize that such further dissection of the hair follicle was unnecessary. (See specification, page 3, lines 22-26; Limat Decl. ¶ 5). This new

methodology has served to simplify the handling process, has reduced the risk for contamination, and has resulted in more efficient initiation of keratinocyte cell plating. (See specification, page 3, lines 26-28; Limat Decl. ¶ 5).

Second, Dr. Limat notes that the methods of the instant invention employ a lower concentration of serum in both the primary and organotypic culturing steps than the methods described in Baur. (See Limat Decl. ¶ 7). Several advantages and benefits to using a lower serum concentration exist, including decreasing the costs involved in production of the epidermal or skin equivalents, decreasing the risk of disease transmission, and improving the state of the cells in the equivalents. (See Limat Decl. ¶ 8-11). Specifically, at higher serum concentrations (such as those described in the examples of Baur), less stratification within the equivalents (which is undesirable) is observed. (See Limat Decl. ¶ 10-12). According the Dr. Limat, at serum concentrations of less than 5%, better stratification and a more normalized, stratified epidermis situation is observed in equivalents prepared according to the methods of the invention. (See Limat Decl. ¶ 10-12).

Third, Dr. Limat acknowledges that, at serum concentrations of less than 5%, keratinocyte precursor cells are seeded at a density of 3×10^4 to 1×10^5 cells cm^2, which is the optimal innoculation density for preparing epidermal or skin equivalents according to the claimed invention. (See Limat Decl. ¶ 13). At other culture densities, more time is required for the cell culture to reach confluence, which is required for exposure of the cells to the air interface. (See Limat Decl. ¶ 13).

Thus, the combination of these elements yields epidermal or skin equivalents that are superior to those disclosed in Baur. (See Limat Decl. ¶ 14). Specifically, as acknowledged by Dr. Limat, epidermal or skin equivalents prepared according to the instant methods can be prepared more quickly, more efficiently, and with less expense than those prepared according to the methods of Baur. (See Limat Decl. ¶ 6, 8, 13).

The addition of Lenoir and Lenoir-Viale to the disclosure of Baur would not lead one of ordinary skill in the art to produce epidermal or skin equivalents possessing the improved properties and characteristics of the equivalents prepared according to the methods of the instant invention. Thus, for all of the foregoing reasons, Applicants contend that the instant claims, as

amended herein, are not obvious over Baur, either alone or in combination with Lenoir and Lenoir-Viale.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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Version with Markings to Show Changes Made

24. (Amended) A method for the selection of keratinocyte precursor cells from the outer root sheath of hair for subsequent use in a composition for healing a skin defect, comprising the steps of:

(a) plucking of an anagen or growing hair;

(b) primary-culturing the outer root sheath-derived keratinocyte precursor cells by adhering said anagen hair, *in toto*, to a microporous membrane, which possesses growth-arrested limited feeder cells on its undersurface so as to select for keratinocyte precursor cells from the outer root sheath of hair, wherein the primary culture medium contains human serum in a concentration less than 5%;

(c) organotypically-culturing the outer root sheath cells harvested from said primary cultures by inoculating a microporous membrane which also possesses growth-arrested limited feeder cells on its undersurface, wherein the organotypic culture medium contains human serum in a concentration less than 5% and the keratinocyte precursor cells are seeded at a density of between 3×10^4 cells cm^2 and 1×10^5 cells/cm^2;

(d) generating an epidermal or complex skin equivalent, for subsequent use as a graft insert, comprised of keratinocyte precursor cells by placing a carrier membrane on top of said organotypic-culture from step (c) and detaching said skin or epidermal equivalent, which is comprised of the keratinocyte precursor cells and carrier membrane, together as a single, laminar unit, and

(e) contacting said epidermal or skin equivalent with a skin defect present on an individual, and immobilizing said epidermal or skin equivalent at the site of contact.

27. (Cancelled)

31. (Cancelled)

35. (Amended) The method of claims 24 or 33, wherein said epidermal equivalents are coated on their top or cornified side with a fibrin glue.

37. (Amended) The method of claim 24, wherein said microporous membrane is coated by one or [ore] more extracellular matrix substances selected from a group consisting of: fibrin, fibronectin, collagens, laminins and hyaluronan.